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(54) Title of the Invention: An Active Oxygen Elimination Agent [derived] from Legumes

(57) [Abstract]

[Objective] To provide an active oxygen elimination agent, using legumes as the raw materials, that is safe and inexpensive and that can be used in broad range of fields such as medicinal drugs, food products and cosmetic products.

[Structure] An active oxygen elimination agent characterized in that it is an aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them.

[Claims]

[Claim 1] An active oxygen elimination agent characterized in that it is an aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them.

[Detailed Description of the Invention]

[0001]

[Field of industrial use] This invention relates to an active oxygen elimination agent that is safe and inexpensive and that can be used in a broad range of fields such as medicinal drugs, food products and cosmetic products characterized in that it is an aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them.

[0002]

[Prior art] When a person has a healthy body, the active oxygen in the body and SOD (superoxide dismutase), which is the active oxygen elimination enzyme in the body are usually in balance and the concentration of active oxygen is maintained at an essentially fixed level. However, production of SOD is decreased due to dietary imbalance, excess stress and aging. On the other hand, active oxygen is increased by smoking and air pollution.

[0003] As a result, active oxygen is present in the body in excess and brings about various kinds of tissue damage. Among the elderly in particular, impairments such as rheumatoid arthritis and Paget's disease are brought about as a result of a decrease in SOD activity and increase in active oxygen concentrations. In addition, the lipid peroxidases that are produced by active oxygen are the principal causes of such modern diseases as myocardial infarction, stroke, cataract, liver spots, freckles, wrinkles, diabetes mellitus, arteriosclerosis, stiffness of the shoulders and sensitivity to cold.

[0004] In the elderly, active oxygen is particularly readily produced in sites such as the skin that are directly affected by environmental factors such as ultraviolet rays. For this reason, production of the pigment melanin and lesions such as liver spots and small wrinkles readily tend to occur accompanying an increase in active oxygen concentration.

[0005] Accordingly, attention has been drawn to SOD, which eliminates the excess active oxygen that is the basis of the various lesions described above and attempts have been made to produce SOD as a medicinal drug product and to add it to cosmetic products and food products for the purpose of preventing or treating these lesions. However, because SOD is unstable in the presence of heat and is deactivated when it is administered orally, and, further, because it is expensive, success has not yet been achieved in elimination of active oxygen by means of SOD.

[0006] On the basis of the circumstances described above, research was conducted on active oxygen elimination agents (substances that contain antioxidants that act in the same way as the enzyme SOD). An active oxygen elimination agent based on raw drug extracts was developed. However, it is made of special raw materials and is expensive. At present, it has not been possible to supply a stable substance.

[0007]

[Problems the invention is intended to solve] Since the time that various lesions attributable to active oxygen as described above were found, there has been a great deal of research with the objective of eliminating active oxygen in the body. Facing an aging society as we are, there is the desire for people to pass their old age in a healthy state. Attention has also been draw to active oxygen elimination agents from a cosmetic standpoint.

[0008] Therefore, there is the desire for the development of an active oxygen elimination agent that is safe for human subjects, that is inexpensive, that is of superior effectiveness in the elimination of active oxygen that brings about various lesions, that can be manufactured simply and for which there can be a stable supply.

[0009]

[Means for solving the problems] The inventors conducted research on various plant components from the standpoint of the harmonization of plants and animals. In the course of this research, it was ascertained that there were many possibilities and effects that had not been anticipated up to the present in legumes, as represented by soy beans, peas, kidney beans, red beans and fava beans. Accordingly, we took up as our research theme the legumes, which have been used as food throughout the world for a long time and which have been demonstrated to be of the highest safety, and conducted research on the overall utilization of legumes. One of these themes was to conduct intensive research on an oxygen elimination agents derived from legumes. When determinations were made of the active oxygen elimination effectiveness of extracts or juices of legumes in unaltered form or of substances that contained them, it was ascertained that there was an extremely marked active oxygen elimination effect. This resulted in the perfection of this invention.

[0010] Specifically, this invention is an active oxygen elimination agent characterized in that it is a aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them. By squeezing legumes, making aqueous extracts (including acid and alkali extraction) or by extraction with organic solvents such as alcohol, oxygen elimination agents that are simple, inexpensive and completely safe and that have excellent effectiveness as described above can be obtained. The legumes that are used as raw materials may be soy beans, peas, kidney beans, red beans and fava beans. Further, the legumes may be immature fresh legumes, completely mature fresh legumes or mature legumes that have been treated by drying.

[0011] When the legumes are made as aqueous extracts or organic solvent extracts, surface area is increased when the legumes are first pulverized or made into powders, for which reason there is extremely good extraction efficiency. The general method is to use a pulverizer. However, they may also be used without pulverizing them. In this case, a long time is necessary for decomposition and extraction of the legume tissues.

[0012] In aqueous extraction, the legumes may be left in unaltered form, or, preferably, they are pulverized and made into a powder and water is added. Extraction can be performed efficiently with a quantity of water added of 2 to 5 times the volume of the legumes. However, the quantity may be selected appropriately depending on yield, workability and the final objective of use. Following that, the materials are heated and extraction is completed at the point in time that a state of boiling is reached. After extraction has been completed, a clear extract is obtained when squeezing or filtering is performed, depending on the objective of use. Extraction may also be performed by adding boiling water at the outset.

[0013] Although the effective component in the extract solution has not been clarified, it has been confirmed that this unknown component is stable in the presence of heat. Therefore, the extraction temperature can be high and thus is efficient. Extraction can be performed sufficiently if the materials are allowed to stand for a long time. However, at temperatures below 40°C, it is necessary to make the pH either acidic or alkaline or to add a preservative. Extraction time may be several minutes in extraction by boiling. At lower intermediate temperatures, several hours to twenty-four hours is necessary.

At low temperatures, several days to one month is necessary due to the pulverized state of the legumes. However, in this case, heating as much as possible is finally more effective.

[0014] The greatest problem in aqueous extraction of legumes in which the principal component is starch is the phenomenon of gelatinization. If gelatinization occurs, there is poor extraction efficiency and there are extremely great difficulties in practical operations. An efficient extraction method in aqueous extraction is to perform a pretreatment with an acid or alkali or to perform a pretreatment by reacting an enzyme that acts on the tissues of the legume (for example, cellulase or lipase). The reason for doing this is thought to be that the effective component is made more easily extractable by the pretreatment.

[0016] It was also ascertained that extracts having this effect are processed in organic solvent extraction as well. In addition to advancing clarification of the effective component, this is also extremely effective for such uses as extracting the effective component under difficult circumstances and in compounding it with substances that are not soluble in water. In this case, it is preferable for it to be finely pulverized as much as possible or to be made into a powder. It is desirable that the organic solvents that are used here be substances such as alcohol that are safe when administered to human beings.

[0017] Squeezing out the juice of legumes has been effective for collection of the effective component from legumes. When juice is squeezed from legumes, ordinary methods may be used such as grinding immature fresh legumes, mature legumes or legumes that have been subjected to heat treatment, enclosing them in a cloth bag and pressing them or squeezing them using a squeezing machine. In this case as well, it has been ascertained that there is an active oxygen elimination action, although the effect is weak, as a result of further subjecting the residue to aqueous extraction or organic solvent extraction after squeezing has been completed.

[0018] There are equivalent effects when fermentation such as alcohol fermentation or lactic acid fermentation is used in combination. Extraction of the product of this invention from legumes is more effective when organic solvent extraction or aqueous extraction is performed as described above and when the effective component in the extract is further subjected to solvent extraction. However, considering that the product is obtained in a concentrated state in this process, equivalent effects can be obtained by concentration.

[0019] Sugar and dextrin are present in the juices and extracted material. Because they are viscous, there are difficulties in obtaining effects depending on the intended use. In this case, the sugar may be removed by having it consumed by yeast, by fractionating the effective component with an adsorbent or by extraction with an organic solvent. In any case, effects appear when extraction is performed, and, depending on the intended use, the unnecessary components may be removed by various methods.

[0020] Legumes have been used every day as foods for a long time and they are so familiar to us that we may not have considered the concept of using then as active oxygen elimination agents. In addition to being eaten in their form as legumes, they are also used in various processed forms such as *tofu* [bean curd], dried bean curd, fried bean curd, bean jam, soybean flour, fermented soy beans, *miso* and soy sauce. However, the concept of and method for extraction of legumes have not been adopted. We believe that the reason for this is that starch legumes become gelatinous when extraction is performed by heating and that, conventionally, extraction was thought to be extremely difficult. For this reason, in this invention the objective can be achieved by facilitating extraction by the action of amylase when organic solvent extraction, acid or alkali extraction or aqueous extraction is performed.

[0021] In the case of legumes such as soybeans in which the principal components are proteins, extraction has been performed conventionally using a method in which soybean milk is collected. However, this is used solely as a nutritional source and has not been used as an active oxygen elimination agent. In addition, essentially no active oxygen elimination action has been found for soybean milk. Specifically, it appears that the objective can be obtained by performing treatment with enzymes such as cellulase and lipase that act on the tissues of legumes or treatments using acids or alkalis as pretreatments. Thus, the effective components can be extracted as superior active oxygen elimination agents by performing sufficient extraction operations on the raw materials.

[0022] We shall now describe the active oxygen elimination effect of the products of this invention. First, we investigated their effects as superoxide elimination agents by various potato operational methods. The test method used was the NBT method.

[0023] Preparation of reagents

1) 0.05 M Na_2CO_3 buffer solution (pH 10.2)

2) 3 mM xanthine solution; 45.64 mg of xanthine was dissolved in the buffer solution to make 100 ml.

3) 3 mM EDTA solution; 111.7 mg of EDTA·2Na was dissolved in distilled water to make 100 ml

4) BSA solution; 15 mg of Bovine Serum Albumin (manufactured by Sigma) was dissolved
in distilled water to make 10 ml.

5) 0.75 mM NBT solution; 61.32 mg of NBT (nitroblue tetrazolium) was dissolved in distilled water to make 100 ml.

6) Xanthine oxidase solution; Xanthine oxidase was diluted with distilled water and its absorbance in a blank test by an operational method (analytical method) to be described subsequently was adjusted to be in the range of 0.2 to 23.

7) 6 mM $CuCl_2$ solution; 102.29 mg of $CuCl_2 \cdot 2H_2O$ was dissolved in distilled water to make 100 ml.

[0024] Operational methods

1) 2.4 ml of Na_2CO_3 buffer solution was collected in a test tube and amounts of 0.1 ml of xanthine solution, EDTA solution, BSA solution and NBT solution were added to it.

2) Next, 0.1 ml of reagent solution was added and the mixture was allowed to stand for 10 minutes at 0°C, after which 0.1 ml of xanthine oxidase solution was added, the mixture was stirred rapidly and was then incubated at 25°C.

3) After 20 minutes, 0.1 ml of $CuCl_2$ solution was added to stop the reaction and absorbance was determined at 560 nm.

4) For the purpose of comparison, the same procedure was carried out on 0.1 ml of an aqueous solution of superoxide dismutase (Cu, Zn type SOD; activity of 3000 to 4000 units/mg; Wako Junyaku) in place of the sample. The value was expressed taking the superoxide elimination rate as 100.

5) The same procedure was carried out using distilled water in place of the sample as a blank. The results of the determinations are shown in Table 1.

[0025]

[Table 1]

	Red Beans		Broad beans		Soybeans		SOD
	Aqueous extraction	Organic solvent extraction	Aqueous extraction	Organic solvent extraction	Aqueous extraction	Organic solvent extraction	
Super oxidase (SO) elimination rate (%)	88	95	89	63	66	50	100

Note 1 The red bean extract used was the product of this invention that was obtained in Example 1.
Note 2 The soybean organic solvent extract used was the product of this invention that was obtained in Example 2.

[0026] As shown above, superoxide elimination effects were found for both the aqueous extracts and organic solvent extracts. It was further ascertained that these effects were essentially the same effects as when alcohol fermentation and lactic acid fermentation are performed.

[0027] Next, a study was conducted of the heat stability of the products of this invention. First, the products of this invention obtained in Example 1 and SOD were heated for 10 minutes at 90°C and the superoxide elimination rates were studied. Determination of the superoxide elimination rates was performed by the method described above. The results are shown in Table 2.

[0028]

[Table 2]

	Red bean aqueous extract	Broad bean aqueous extract	Soybean aqueous extract	SOD
SO elimination rate (%)	89	88	65	0

Note: The extracts used were those obtained in the respective examples.

[0029] As shown above, it was found that SOD was unstable in the presence of heat, whereas the products of this invention exhibited superior thermal stability. On the basis of this finding, it can be said that the effective component of the product of this invention that eliminates active oxygen is of superior stability in the presence of heat. Because the products of this invention exhibit marked active oxygen eliminating effects, and, moreover, are safe, they can be used in medicinal drugs, cosmetic products and food products. Next, we shall describe their uses.

[0030] As medicinal drug products, they can be used as antiulcerative agents. Next, we shall present the experimental methods whereby the antiulcerative action of the products of this invention is studied and the results of these experiments. A study was conducted of the action of products of this invention when administered orally in restrained water immersion stress ulcers. This was performed in accordance with the method of Watanabe et al.. Specifically, eight week old male ddY strain mice were fasted for 24 hours, after which 0.3 ml/mouse of the product of this invention obtained in Example 1 was administered orally. After 30 minutes, the mouse was placed in a stress cage and was immersed in water at 15°C up to the xiphoid process and was subjected to a restrained water immersion test. After 5 hours, the mouse was killed by dislocation of the cervical vertebrae and the stomach was excised. Following that, 1.5 ml of 1% formalin solution was injected into the stomach. In addition, the stomach tissues were gently fixed by immersion in this same solution and were allowed to stand for 24 hours. Following that, an incision was made along the greater curvature of the stomach and the lengths (mm) of the lesions that had been generated in the gastric glands region were determined. The sum per animal was expressed as the ulcerative coefficient. Animals given oral administration of the same quantity of physiological saline solution 30 minutes before introduction into stress cages were used as controls. Fifteen mice were used in each group. The results were summarized in Table 3.

[0031]

[Table 3]

	Dose (ml)/mouse	No. of samples	Avg. of ulcerative coefficients
Physiological saline solution	0.3	15	65.8
Product of this invention	0.3	15	34.9

[0032] As shown in Table 3, the average of the ulcerative coefficients in the mice that had been given physiological saline solution as a control was 65.8, whereas the average of ulcerative coefficients in mice to which the product of this invention had been administered was 34.9. Thus, it was clearly ascertained that the product of this invention was effective as an antiulcerative agent against restrained water immersion stress ulcers on oral administration. As a result, it was ascertained that products of this invention act directly from the gastric mucosa and exhibit an effective action as antiulcerative agents.

[0033] Next, products of this invention can be used as skin treatment agents. Products of this invention were applied twice a day, in the morning and evening, to a panel of patients suffering from various skin diseases, and these treatments were continued for one month. The diagnosed results are shown in Table 4.

[0034]

[Table 4]

	Marked Improvement	Useful	Somewhat Useful	Undecided Which	Discontinued	Usefulness (%)
Scratches, cuts	0	3	5	4	0	66.7
Burns	1	3	5	2	0	81.8
Diaper rash	0	3	6	2	0	81.8
Insect bites	0	1	4	2	0	71.4
Eruptions, pimples	0	5	9	4	0	77.8
Blackheads	2	3	5	2	0	83.3
Chapping, cracking	0	3	3	2	0	75.0
Xeroderma	1	2	8	2	0	84.6
Itching of skin	0	2	12	5	0	73.7
Eczema	1	4	6	3	0	78.6
Atopic dermatitis	1	5	4	6	0	62.5
Vesicular mycotic infections	0	3	4	1	0	87.5
Keratinized mycotic infections	0	4	2	1	0	85.7

(Note) 1 The product of this invention obtained in Example 1 was used.

(Note) 2 Usefulness is the overall percentage of marked improvement + useful + somewhat useful

(Note) 3 Evaluations were made by specialist physicians.

(Note) 4 The panel consisted of a total of 68 patients, 35 males and 33 females. The average age was 32.5 years (age 1 to 79).

[0035] As shown in Table 4 above, because this product was effected as a therapeutic agent for diverse skin conditions, it was concluded that is has a fibroblast activating action and that it also has an antimicrobial action. Further, because it was useful in xeroderma and blackheads, it was concluded that it has a humectant action and an action that inhibits increase of sebum to a suitable degree. The experimental method whereby this humectant action and action in inhibiting the increase of sebum to a suitable degree was studied and the results were as follows.

[0036] First, in order to illustrate the intensity of the humectant action of this invention, a single application experiment was performed using a moisture meter (SKICON 200). The determination conditions were an environment set to a room temperature of 20°C and a relative humidity of 65%. The members of the panel were allowed to rest in this environment for about 10 minutes before the determinations. The test sites that were selected were (bilateral) sites on the forearm on which exanthema was not found. The members of the panel were 5 individuals who were suffering from xeroderma. Figure 1 shows the average values for changes in the water content of the stratum corneum as read from the moisture meter in this experiment (in which the product of this invention obtained in Example 1 was used) and in the control experiment (in which water was used). The method of determination in the single application experiment is described below.

[0037] Determination method

1) Test sites and control sites of 5 × 5 cm were established on the forearms of the panel members.

2) The water content of the stratum corneum in these sites was determined.

3) Determinations were made of the water content of the stratum corneum immediately after application of the test material and after 30, 60, 90 and 120 minutes.

[0038] From Figure 1, it can be seen that the water content of the stratum corneum immediately after application of the product of this invention was approximately 8 times greater than that in the controls. When the findings from 30 minutes up to 120 minutes after application are examined, it can be seen that water content in the sites at which the product of this invention was applied were maintained at levels 2 to 3 times that in the controls up to 120 minutes.

[0039] Next, in order to provide numerical corroboration of the therapeutic effects of the product of this invention in xeroderma, water load experiments were performed using a moisture meter (SKICON 200) before use of the product of this invention and after two weeks of use. The panel consisted of the five individuals used for Figure 1 and the same determination conditions were used as in the single application experiments. A control group (in which determinations were made for sites at which the product of this invention was not applied) had to be established so that seasonal changes in the water content of the stratum corneum in vivo would not affect the evaluations of effectiveness. The water content of the stratum corneum was shown as an average value for the five panel members. The results are shown in Figure 2. The product of this invention that was used was the product obtained in Example 1. The method of determination in the water load experiment is described below.

[0040] Determination method

1) The water content of the stratum corneum was determined at the test site.

2) One drop of distilled water was placed on the test site and the water drop was completely wiped off with dry gauze after 10 seconds.

3) Water content of the stratum corneum was determined immediately after wiping and after 30, 60, 90 and 120 seconds.

[0041] As shown in the graph of Figure 2, improvement was found at the same time in the water absorption capacity of the skin (which was found by subtracting the value for water content of the stratum corneum before loading from the water content of the stratum corneum at

0 seconds after water loading) and in its water retaining capacity (the curve traced for water content of the stratum corneum from 0 seconds up to 120 seconds after water loading).

[0042] Specifically, before use of the product of this invention, the water content of the stratum corneum of the skin before water loading was extremely low (average of 4.6) and water absorption capacity (average of 42.0) was quite low. In addition, in the study of water retention capacity, the water absorbed in the stratum corneum of the skin of normal persons gradually decreased, with a return to values before water loading being seen 30 seconds after water loading. These results indicate that water absorption capacity, water retention capacity and barrier functions all decreased in the diseased stratum corneum for which determinations were made. By contrast, after use of the product of this invention, both the water content and the water absorption capacity of the stratum corneum of the skin increased to more than twice that before water loading and water retention capacity was also considerably improved so that it was essentially no different from that of normal individuals.

[0043] On the basis of these findings, it can be said that the product of this invention has a superior action in improving the state of water content and the barrier function of diseased stratum corneum. When the products of this invention are evaluated taking into consideration the humectant action obtained in the single application experiments, it can be said that the products of this invention increase the water absorption capacity and water retention capacity of the stratum corneum, that they absorb large quantities of water from the outside and that they have a humectant action that confers on the stratum corneum the property that it does not release water once it has been absorbed.

[0044] Further, in order to provide experimental verification of the inhibitory effect of products of this invention on secretion of sebum, determinations were made in changes in the quantity of sebum after washing of the face. The panel consisted of five individuals selected at random from the group used for Table 4. Figure 3 shows the average values for changes in the quantity of sebum in this experiment (application of the product of this invention after washing the face) and in the control experiment (washing of the face only). The product of this invention that was used was that obtained in Example 1.

[0045] As shown in the graph in Figure 3, when the product of this invention was applied, it was ascertained that an increase in the quantity of sebum was considerably inhibited. The prophylactic and therapeutic effects in blackheads were also supported by the inhibitory effects of the products of this invention on secretion of sebum. Further, when products of this invention were applied to the skin as cosmetic products, it was ascertained from the following experiments that there was a smoothing effect on the texture of the skin, that there is a wrinkle stretching rejuvenating effect and an aging preventing effect.

[0046] The product of this invention was applied twice a day for one month to sites on the right arms of the members of the panel and determinations of the sites of application of the product of this invention were made with a kinematic friction meter. The same sites on the left arm were used as controls. The panel consisted of six members.

The determination conditions are described below.

Temperature: 25°C
Humidity: 60°
Sensor used: KES-SE friction sensitivity tester SE-2 type (0.5 mm piano wire used)
Friction static load: 50 gf
Determination speed: 1 mm/sec
Determination distance: 30 mm (integrated effective range, 20 mm)

[0047] The MMD (coefficient of variation) for the sites on the left arm to which the product of this invention was not applied was 0.0186. However, the MMD (coefficient of variation) on the sites on the right arms to which the product of this invention had been applied for one month was less than 0.0084. The average values for the six subjects were essentially the same. It is thought that this was because there was little variation due to irregularities of the surfaces. From this finding, it was ascertained that the skin had become smoother and that there was stretching of wrinkles and rejuvenation. When MIU (coefficient of friction) was studied at the same time, it was 0.138 before application and less than 0.102 after application for one month. Thus, it was ascertained that there was a smoothing effect on the skin, a softening effect on the skin and that there was also an aging preventive effect.

[0048] In order to verify the beautifying effect of this invention, an experiment was conducted on its inhibitory action on tyrosinase. As the operational method, amounts of 1 ml each of substrate solution (0.04% tyrosine solution) and of buffer solution (McIlvaine Buffer, pH 6.8) were precisely collected in an absorption cell, amounts of 1 ml of water and of the product of this invention obtained in Example 1 were precisely introduced and the materials were mixed by stirring. After 5 minutes, the absorbance scale was zero corrected in alignment with a wavelength of 475 nm. Next, 0.02 ml of tyrosinase solution (obtained by dissolving 5.3 mg of tyrosinase in 0.9% NaCl solution) was precisely added. The mixture was immediately stirred and then incubated. The absorbance at that time was determined over time (at 3 minute intervals).

[0049]

[Table 5]

Time in Minutes	Water	Product of this Invention
0	0.011	0.012
3	0.058	0.045
6	0.152	0.059
9	0.243	0.097
12	0.316	0.099
15	0.414	0.118
18	0.498	0.133
21	0.552	0.142
24	0.621	0.151
27	0.623	0.157
30	0.629	0.162

[0050] From the results of the determinations shown in Table 5, it can be seen that there was a tyrosinase activity inhibiting action. On this basis, it can be said that the products of this invention have a beautifying action.

[0051] Further, as stated above, the products of this invention have a humectant action to the extent that they can be used as medicinal drug products. Consequently, they have actions that are basic for cosmetic products and have wide uses as creams, emulsions, toilet water, cleansers, packs and soaps. Effects similar to those described above can also be obtained by drinking the products of this invention.

[0052] The products of this invention can be used as preservatives of food products and as agents for maintaining freshness. We next conducted tests of the antibacterial activity of the products of this invention against Bacillus subtilis and Bacillus cereus, which cause spoiling of cooked rice and bread, as representative gram-positive bacteria, and against Escherichia coli, which is an index of general contamination, as a representative gram-negative bacterium. The results are shown in the table.

[0053] An amount of 1 ml of the product of this invention was added to 10 ml of ordinary agar culture medium as the culture medium. Culture medium to which 1 ml of water was added instead of the product of this invention was used as the control. Culturing was performed for 48 hours at 37°C and the state of growth of each bacterium was observed. The results are shown in Table 6.

[0054]

[Table 6]

	Red bean (azuki] aqueous extract	Soybean aqueous extract	Water
Bacillus subtilis	–	–	+++
Bacillus cereus	–	–	+++
Escherichia coli	–	+	+++

Note 1 Evaluations: - : no growth; +: small amount of growth; ++ growth; +++: large amount of growth

Note 2 The red bean aqueous extract used was that obtained in Example 1 and the soybean aqueous extract used was that obtained in Example 2.

[0555] As should be evident from Table 6, in the culture media in which water was added as a control there was fairly extensive growth of both food spoilage bacteria and Escherichia coli on culturing for 48 hours at 35°C. By contrast, although there was slight growth of Escherichia coli in culture media to which the product of this invention was added, no growth whatsoever of bacteria of the genus Bacillus was found. From these results, it was ascertained that products of this invention have extremely strong antibacterial effects.

[0056] Next, a study was made of the effects of the products of this invention in inhibiting formation of oxides by the iron rhodanide method. Specifically, a study was made of the effects of the products of this invention in inhibiting oxidation of linoleic acid, which is extremely readily oxidized. The determination method is described below.

[0057] Preparation of reagents

1. 0.2 M phosphate buffer solution (pH 7.0)

2. 2.6% ethanol lineoleate solution

3. 75% ethanol solution

4. 30% ammonium thiocyanate

5. 35% hydrochloric acid solution of 0.02 M of ferric chloride

[0058] Operational methods

1. 0.1 ml of test material solution, 0.1 ml of 0.2 M phosphate buffer solution, 0.5 ml of water and 0.2 ml of 2.6% ethanol lineolate solution were added and were thoroughly mixed, after which the mixture was allowed to stand for 5 days at 37°C.

2. 50 µl of oxidation treatment solution , 4.85 ml of 75% ethanol solution, 50 µl of 30% ammonium thiocyanate and 50 µl of 35% hydrochloric acid solution of 0.02 M ferric chloride solution were mixed and the absorbance of the mixture at 500 nm was determined after 5 minutes.

3. A blank was made by the same procedure using distilled water instead of the sample. The results are shown in Table 7.

[0059]

[Table 7]

Name of Sample	A_{500}	Oxidation percentage (%)
Water (control)	0.352	100.0
Red bean aqueous extract	0.064	18.2
Soybean aqueous extract	0.047	13.4

Note 1 The red bean aqueous extract used was the product of this invention that was obtained in Example 1

Note 2 The soybean aqueous extract used was the product of this invention that was obtained in Example 2.

[0060] As should be evident from Table 7, it was ascertained that the product of this invention has a superior antioxidant effect for lineoleic acid, which is extremely easily oxidized.

[0061] Thus, because the products of this invention are safe for human beings, have antibacterial effects against diverse bacteria, have a browning preventing effect and have an antioxidant effect, they can be widely used for food products as preservatives and as antioxidants and agents for maintaining freshness.

[0062]

[Effect of the invention] As should be evident from the data described above, active oxygen elimination agents that are simple, completely safe and stable, that have a superior active oxygen elimination effect and that have other diverse effects can be obtained by subjecting legumes to aqueous extraction, organic solvent extraction or squeezing.

[0063] Because legumes have long been used as foods, almost no methods of manufacture or uses have been developed in new fields apart from their use as foods. Further, because legumes have been used as foods, their safety has been demonstrated.

[0064] Consequently, it was discovered that this invention can be used as a medicinal drug product for the prevention or treatment of the diseases indicated above, that it can be added to foods and cosmetic products and can play a role in promoting health and for purposes of beauty and that active oxygen elimination agents that can be used in a broad range of fields can be obtained simply from familiar legumes the safety of which has been demonstrated.

[0066] Up to the present, legumes have been used only as foods. The fact that mew uses for legumes have been developed and that the new possibilities have been discovered for them is of extremely great significance.

[0066]

[Examples] Example 1

1 kg of mature dry red beans was thoroughly pulverized, 3 liters of warm water at 55°C and 15 g of liquefaction enzyme were added to them and the mixture was thoroughly stirred. Following that, it was gradually heated and extraction by boiling was performed for 5 minutes, after which it was cooled to 30°C. Following that, it was squeezed with a squeezing machine, with 2.5 liters of red bean aqueous extraction solution and 1.4 kg of residue being obtained.

[0067] Example 2

1 kg of mature soybeans was thoroughly pulverized, 2 liters of 90% alcohol were added, the mixture was thoroughly stirred and was then allowed to stand for 24 hours, after which it was squeezed with a squeezing machine, with 1.5 liters of pressed solution and 1.3 kg of residue being obtained. 2.0 liters of water were added to the pressed solution, it was concentrated under reduced pressure, the ethanol was removed and 1.4 liters of product of this invention were obtained.

[0068] Example 3

3 kg of green soybeans were introduced into a pressing machine and 0.6 liter of juice and 2.4 kg of residue were obtained.

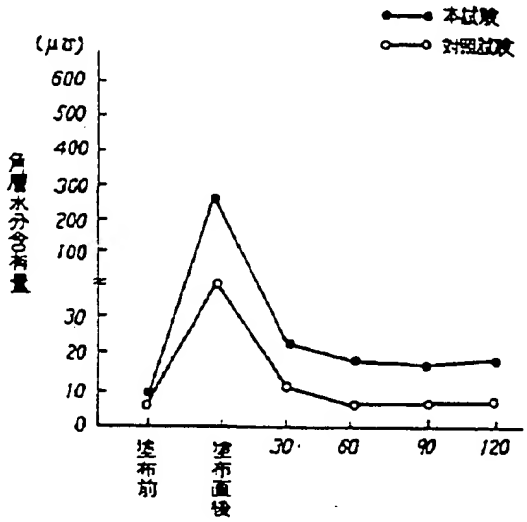
[Brief Explanation of the Figures]

[Figure 1] This is a graph that shows the results for the moisture maintaining effect of a product of this invention and water when single application experiments were performed using a moisture meter (SKICON 200).

[Figure 2] This is a graph that shows the results when water load experiments were performed before use of the product of this invention and after two weeks of use.

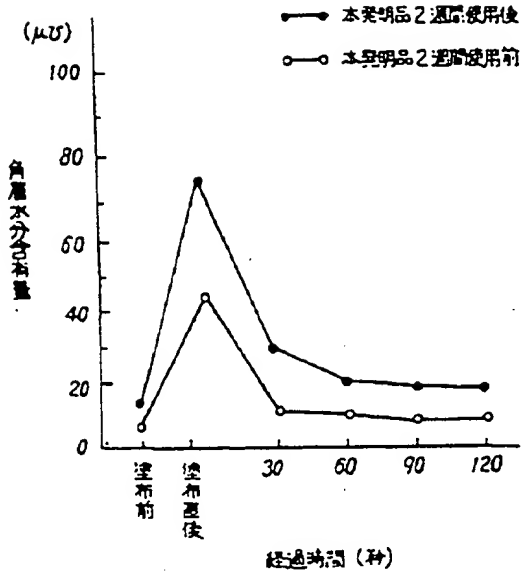
[Figure 3] This is a graph that shows the results of experiments on changes of sebum content in cases in which the product of this invention was applied after washing of the face and in cases of face washing only.

[Figure 1]



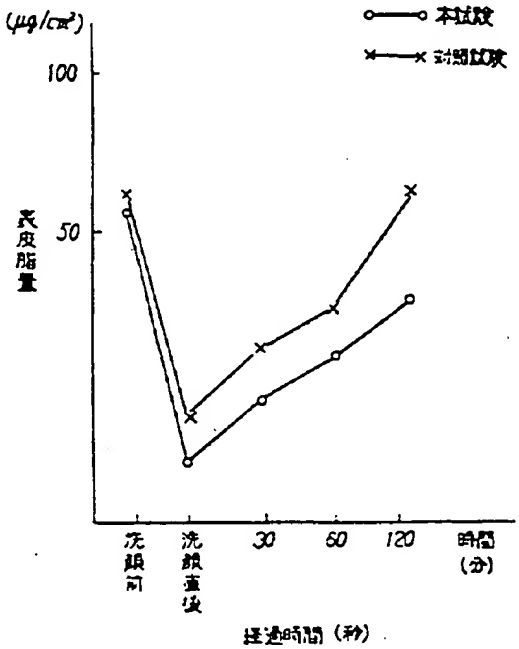
[vertical axis]: Water content of stratum corneum
[horizontal axis: horizontal characters]: Course over time (minutes)
[horizontal axis: vertically written character groups, left to right]:
Before application; Immediately after application
[inside graph at upper right]
This experiment
Control experiment

[Figure 2]



[vertical axis]: Water content of stratum corneum
[horizontal axis: horizontal characters]: Course over time (seconds)
[horizontal axis: vertically written character groups, left to right]:
Before application; Immediately after application
[inside graph at upper right]
After 2 weeks of use of product of this invention
Before 2 week use of product of this invention

[Figure 3]



[vertical axis]: Amount of sebum in epidermis
[horizontal axis: horizontal characters]: Course over time (seconds)
[horizontal axis: vertically written character groups, left to right]:
Before washing face; Immediately after washing face
[inside graph at upper right]
This experiment; Control experiment